

PROGRESS REPORT
(March 31, 1967)

NASA Grant No. NGR 21-002-040

MOLECULAR BINDING IN THE CELL SURFACE

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N67-26284

FACILITY FORM 802

(ACCESSION NUMBER)

(THRU)

25
(PAGES)

5
(CODE)

CR-84051
(NASA CR OR TMX OR AD NUMBER)

04
(CATEGORY)

ABSTRACT:

The microwave absorption of various protein solution, water, and organic solvents was examined with a Tektronix # 1L-30 Spectrum Analyzer in an attempt to determine the feasibility of the application of a new instrument to bound-water studies of cell proteins. Differences were observed in the absorbed microwave power for varying concentrations of Bovine Serum Albumin dissolved in water and for these solutions with and without a dissolved barbiturate. Marked differences were observed between the absorption of water-based solutions, the absorption of gels, and the absorption of organic solvents. Results suggest the validity of the Analyzer application, but the detailed interpretation of spectra and a proper assessment of the limitations of the instrument will require additional experimentation and refinement in the microwave hardware.

The existing instrument is described, and additional microwave equipment needed to improve its precision is discussed. Outlined are specific experiments designed to further elucidate the role of binding mechanisms in the observed absorption. Work currently in progress is reviewed.

Theoretical Considerations:

The absorption of a cavity in the microwave region may be described in terms of its Q , or quality factor. Q is defined in terms of the angular frequency, ω , and the leakage resistance, R_s , of an associated capacitance, C , as

$$Q = (\omega C R_s)^{-1} \quad (1)$$

For $Q > 10$, as Q becomes large its relationship to the frequency at which maximum resonance occurs, f_0 and to f_1 and f_2 , the lower and upper frequencies which locate the half-width of the resonance characteristic, becomes in the limit exactly

$$Q = \frac{f_0}{f_1 - f_2} \quad (2)$$

The introduction of dielectrics into a waveguide broadens its resonance, lowering the Q , and shifts the frequency, f_0 , at which maximum resonance occurs. Thus there arise equations of the Vogelhut type, which determine relative changes in the complex dielectric constant, ϵ^* , in terms of the above factors,

$$\Delta \epsilon' = \frac{2C}{f_0} (f_s - f_w) \quad (3)$$

$$\Delta \epsilon'' = C \left[Q_s^{-1} - Q_w^{-1} \right] \quad (4)$$

The expression compares the dielectric content $\frac{\Delta \epsilon}{\epsilon}$ for water with that for the same volume of a solution in which $(f_s - f_w)$ is the shift in resonant frequency, Q_s and Q_w are for the water and solution, and the cavity constant

C relates the volume of the empty cavity, V, the volume of dielectric material, v, and a nodal correction factor for the position of the dielectric relative to E_{\max} (3)

$$C = V (4 A v)^{-1} \quad (5)$$

In a Debye analysis, ϵ^* yields ϵ_s , the static dielectric constant, which is conditionally valid for dilute solutions and relative measurements. Given these conditions, the Haggis approximation holds for the linear dependence of ϵ_s on the concentration of solute, such that w, the weight of water irrotationally bound to 1 gm. of protein, is given in the relationship, (6)

$$\epsilon_s = \epsilon_w - \beta \left[(\epsilon_w - \epsilon_{\infty p})^v + (\epsilon_w - \epsilon_{\infty w})^w \right] \times \text{gm}_p \times (\text{ml solution})^{-1} \times 10^{-4}$$

β is given by the axial ratio of the ellipsoidal cavity occupied by the macro-molecule, v is the partial specific volume of protein; $\epsilon_{\infty p}$ and $\epsilon_{\infty w}$ are the high frequency dielectric constants of water and protein. (5)

The central question underlying the Spectrum Analyser application is this: how readily can the spectral display of power absorbed vs frequency be related to the above method for estimating irrotationally bound water? The answer to this query is incomplete at this juncture in our work. However, the considerations suggested below lead one to believe that a spectral display contains information which is in every way analagous to information obtained by more conventional instruments; such as graphic readout, oscillographic record and the decrement associated with power absorption meters.

A spectrum analyzer capable of high dispersion has a wide spectral width; that is, it accepts a wide RF pass band at its input. The instrument evaluated here has a selectable spectrum width which is fully capable of displaying the entire Q characteristic of a microwave cavity as Q is imaged in the power absorption near resonance. As frequency departs from the resonant frequency, the display is symmetrical about an ordinate of maximum or minimum signal amplitude. It contains lower and upper points of inflection near the half-width of the resonance characteristic and contains stationary points at larger frequency deviations. In some cases (vis. Fig. 10, upper trace), the display suggests a canonical distribution of the Cauchy form (6). Insofar as the microwave signal is an accurate image of Q, the stored trace may be photographed and examined directly for the frequency at the half-power points. However, this technique may not yield sufficient precision for reliable measurements of small differences in Q, since high values of dispersion necessarily decrease the resolution of the analyzer.

Perhaps a well-known probability distribution of the Gaussian type could be made to fit the absorption curve. Consider a family of these curves, described by the function,

$$P(f) = P_m e^{-\frac{f^2}{a^2}} \quad (7)$$

Since power, P, is thus related to its maximum, P_m , the exponential may be evaluated for $\frac{Pm}{2}$ by knowing the amplitude alone if a is known. The above treatment does not describe a nor consider its variability for a family of curves, which would make P a function of two variables. However, it

suggests a mathematical approach for finding Q from the amplitude of maximum absorption alone for a well-defined function. An advantage in precision may very well accrue, since this maximum may be determined at the superior resolution given by low dispersion settings of the instrument and then converted to power differences from the square law display, which shows a maximum differentiation in the observed signal.

In the same way, the frequency associated with maximum resonance may be determined more accurately with the superior selectivity of the lower frequency settings of the dispersion-coupled resolution control. As before, only a small portion of the cavity absorption characteristic will be displayed with the limited band-pass through the instrument. As a given absorption maximum may well fall in the stop band outside the display, one or more of the controlling parameters will have to be shifted to put the signal on the oscilloscope, and the frequency difference of this shift will have to be known. To date, this has been most easily achieved by shifting the "window" of the analyzer IF within a wider RF pass band. Larger frequency shifts for more gross changes within the cavity are easily observed at high dispersion settings. Some of these are shown in Figs. 9-12. However, no "best" technique has been evolved as the data so far collected is incomplete, imprecise and inadequately controlled. Obviously however, ΔQ and Δf , reliably determined, do yield quantitative estimates of bound water good for relative comparisons, and since both have been observed with the spectrum analyzer alone, the only apparent limitation to its application in this field of biological measurement would be its precision, which has not as yet been completely assessed.

Instrumentation:

Tektronix has recently made available at relatively low cost a series of plug-in spectrum analyzers to fit their oscilloscope. One model, the # 1L30 Plug-in Spectrum Analyzer, has a frequency range to 10.5 GHz. This RF capability suggested to us its application in measuring the microwave absorption of neurobiological preparations. The spectrum analyzer permits the display of signal amplitudes on the vertical plates of the oscilloscope as a function of frequency, which replaces the normal time base. Though the instrument has been in existence since World War II, its cost and complexity have confined it to specialized radar measurements until recently, when advances in SHF circuitry and cost reductions have made broader applications possible.

Figure 7 illustrates a 1L30 Spectrum Analyzer loaned to us through the courtesy of Tektronics, Inc., and Mr. D. Wilson, the regional field engineer. Advantages in its use were immediately apparent. First, it displays the signal at its input almost instantaneously and at a rapid repetition rate. This means that the oscilloscope displays a virtually continuous visual record of what is happening in the waveguide. Second, it has its own frequency sweep capability. The application of a ramp function to the frequency control element of a voltage-tuned oscillator causes a linear display of frequency across the selected RF pass-band⁽⁷⁾. Third, the analyzer properly terminates the transmission line, while at the same time its heterodyning circuit draws virtually no current from its input. Perturbations and measurement errors due to the instrument are negligible. Fourth, linearity problems associated with bolometers and crystal detectors in unstable environmental conditions are avoided.

In contrast, conventional instrumentation requires the use of a graphic X-Y recorder, which must trace slowly due to the ballistic requirements of the pen and be reset after each pass. It requires an externally derived, frequency proportional signal for the X drive, and its Y input must be supplied by the rectified output from a crystal detector located at a reflection termination for the cavity. Apart from problems of generator stability and the possibility of imposing an additional loss transient on the dielectric at rotational frequencies for its dipole, sweeping the LFE oscillator entails a precise mechanical drive with high linearity and minimum lash. The complexity and cost of a regulated power supply, a precise voltage divider, and a mechanical assembly on the oscillator would become unnecessary if the analyzer did the measurements job alone. If not, the incorporation of a recorder output at the instrument would considerably simplify the addition of the graphic recorder.

A record must be made of the cavity signal at the time of measurement to permit the detailed comparative analysis needed in bound water estimates. Certainly a graphic instrument or a more elaborate tape readout would be essential if it were not for the recently developed storage oscilloscope. Photographs of the stored trace taken against superimposed graticle lines may be compared directly for the variations shown. Whereas one might question the precision of this technique for indicating small variations in the entire cavity absorption trace, magnifying the display to include only those small portions of the signal of specific interest would increase not only the visual accuracy but the resolution of the instrument. The superior detail in the photograph of Fig. 8, taken at 0.2MHz/cm dispersion, to Fig. 9, taken at 0.5 MHz/cm, is evident. Analytic techniques appropriate to this procedure have

been suggested above.

The initial instrument arrangement is shown in Figs. 1 and 2. Located on each side of the environmental housing for the cavity are the oscillator and the oscilloscope containing the analyzer. Since the klystron is capable of 0.5 watts output, the waveguide system includes a 10 dB fixed attenuator, Hewlett Packard # X 370C, at the source, as in Fig. 4. This reduces perturbations and affords some measure of isolation for the cavity. The guide enters the housing above a 20 dB, two-directional coupler, Hewlett Packard # X 752D, and terminates at a waveguide to coaxial adapter, Narda # 601A, located beyond an additional 6 dB pad, the Hewlett Packard # X 370B fixed attenuator. This is shown in Fig. 5. In Fig. 6, the cavity, mounted on the floor of the housing, is attached to the two-directional coupler. A small section of # RG-9B/U coaxial cable exits through the side of the housing and connects to the input of the analyzer.

As the # 1L30 spectrum analyzer was housed in a Tektronix # 547 oscilloscope, which does not have storage capability, a slave # 564 oscilloscope was connected to the parent # 547 unit, its input patched to the vertical output and its sweeps triggered externally from the main instrument. All photographs were recorded from the slave oscilloscope in the storage mode. Gaps in the stored trace are due to the age of the slave CRT, which has a limited storage lifetime. Improvements in the stored trace and in resolution would be gained by using the # 549 storage oscilloscope as the parent analyzer instrument.

The microwave hardware falls far short of an ideal system, and a variety of improvements are planned to reduce VSWR and perturbations. 40 dB front-to-back isolators located at the source, cavity, and secondary to the directional

coupler will increase isolation and reduce perturbations considerably. Additional attenuation of the klystron is not only possible but desirable at the analyzer input, since it will avoid the possibility of harmonic mixing, which may occur when the RF signal at the input is > -30 dBm. A phase shifter may improve VSWR. It is to be noted that the analyzer itself has been an extremely useful diagnostic tool for minimizing spurious guide reflections and will probably continue to be the primary instrument used in optimizing the transmission line, if indeed other devices are necessary.

Results:

This section must be prefaced by a brief comment on the preliminary nature of the results shown. They seem to hold much promise for future studies of a more exhaustive nature, but should not be interpreted as reliable quantitative indices of the liquids measured in the microwave cavity. It seems clear that absorption differences do exist among the various liquids for one setting of the analyzer, oscillator, and cavity reflection when the dielectric position near a cavity node is fixed. These differences have not always been observed, but they have been observed consistently when the microwave system was known to be in a refined state of tune. At one temperature, the differences associate in a regular way with the amount of water displaced in a given volume by solute molecules. Changes in the absorption of one protein due to the addition of a barbiturate are in the direction of water alone, indicating a possible decrease in bound water due to its displacement by the barbiturate molecule.

The empty cavity was found to resonate at approximately 8.8 GHz. This resonance was found by tuning the oscillator and the variable short at the cavity for a maximum of the absorption cusp displayed on the trace, as in Fig. 8. If it is assumed that this display images Q accurately, then at the half-power frequency difference,

$$\Delta f = 0.85 + 0.82 = 1.67 \text{ MHz},$$

Q is given by (2) to be,

$$Q = \frac{8.8 \times 10^9 \text{ Hz}}{1.67 \times 10^6 \text{ Hz}} = 5.27 \times 10^3 \quad (8)$$

This value is in good agreement with Birnbaum and Franeau, who found Q 's of the order of 5×10^3 . But confidence in the result must be qualified, since the curvature of the display is not ideal and cavity perturbations were evident.

Since suitable quartz tubing was not immediately obtainable, a glass tube with an o. d. of 0.122" was used to hold the liquid sample in place in the cavity. Its i. d. was determined photographically by double-exposing on the same film the focused microscope image of a Zeiss graticle calibrated in 0.1 mm divisions and the focused cross-section of the tubing. The microphotograph is reproduced in Fig. 15. The diameter may be determined to 0.01 mm, but length-dependent variations in the tube i. d. have not been carefully studied, so that the calculated internal sample volume below is only approximate. However, the indicated diameter is nearly 1.42 mm and the short transverse dimension of the cavity is 0.450 ± 0.143 mm. Thus the volume of sample within the guide is given by, (7)

$$V = 0.25\pi d^2 h = (1.42)^2 \times 10^{-2} \times 1.143 \times 2.5 \times 10^{-1} \text{ cm}^3 = 5.77 \times 10^{-3} \text{ ml.}$$

It is to be noted that other workers have used different internal sample volumes than that shown here. This further complicates any effort to make direct comparisons between our results and theirs, and we hope to replace the sample tube with one directly comparable to that used by Vogelhut, (9) which enclosed 10^{-4} ml in a tube of 0.080 in o. d.

In early experiments with the cavity, a series of sample tubes were made from one size of tubing stock, and these were freely interchanged in the cavity, each tube identified with a particular solution. It was found, however, that even after locking the sample carriage in place, tubes containing identical solutions would not give identical cavity reflections. The tolerances necessary to allow the easy insertion and withdrawal of a tube exceeded the critical axial location requirements relative to a node. It was also evident that differences existed from tube to tube in the internal volume, its location

relative to the glass-air interface, and perhaps even in the composition of the glass. As these difficulties could be obviated by fixing one tube in place in the cavity and circulating fluids into the tube from outside the cavity, the apparatus of Fig. 14 was devised. We have since learned that Vogelhut⁽⁹⁾ adopted a similar procedure in his earlier work. A constant temperature bath will soon be added to the exterior of the cavity housing, with the valving appropriate to circulating a variety of liquids through the tube from the external reservoirs.

Data for the observed frequency shifts must be qualified by the same limitations that have applied to other results, and it must be specifically noted that shifts associated with maximum resonance have not been determined. Gross changes in frequency due to the introduction of the glass tube are associated with a cusp displacement of 3.75 MHz in the analyzer trace, as in Fig. 9. This shift is not the actual shift in cavity resonance due to the dielectric. It can be interpreted only to indicate that a frequency shift is associated with the appearance of glass tubing in the cavity, in accordance with theoretical considerations. The frequency shifts due to water, glycerol, and protein solutions, shown in Figs. 10, 11, and 12, must be interpreted in the same way. In Fig. 13, an attempt was made to delineate the slight difference in the trace between water and a dilute egg albumin solution at broad band-pass analyzer settings. The photograph did not reproduce well, but the shift was in any case hardly discernable, and it indicates that the greater selectivity and sensitivity given by the choice of narrow pass-bands in the RF and IF sections of the instrument would be necessary to discriminate between nearly similar solutions. As is clear from the data, information so far accumulated is only

a beginning, and its association with absorption maxima is only now being attempted.

It was found that when wider pass-bands were chosen, the immodulated signal of the microwave oscillator produced on the display of the analyzer a principal ordinate and then a series of subsidiary ordinates at different frequencies, as the klystron output exceeded certain power levels. It seemed evident that the secondary ordinates were harmonically related to the fundamental one, although there has not been time to establish this mathematically.

Tektronix describes a similar phenomenon as due to harmonic mixing in the spectrum analyzer when the input is overdriven, and they consider the harmonic
(10)

products to be spurious. . When the analyzer sensitivity was up-graded by narrower-band dispersion and resolution settings, the principal ordinate was found to drift across the display with even slight temperature changes and its position on the trace was found to vary sporadically when the oscillator was not in the stabilized mode. On the other hand, when the cavity housing was sealed up to effect some temperature stability and when the oscillator was tuned to the stabilized mode, ordinates appeared regularly at the same position. In Fig. 17, the ordinates represent the summation of 150 individual spikes for each of the various solutions.

At one axial location of the sample tube in the cavity, the ordinate displayed was a relative minimum. If the tube was shifted to either side of this location, both frequency and amplitude shifts occurred. The same shifts were seen when the variable short at the reflection end was moved inward or outward from this minimum. The results of several passes through this node with water in the sample tube are graphed in Fig. 22, in which planes of the frequency

and amplitude shifts are projected as functions of their axial travel. Some remarkable similarities occur between these curves and those which display absorption as a function of frequency in the analyzer. The total frequency shift in the X-Y plane is $5 \times 0.5 \text{ MHz/cm} = 2.5 \text{ MHz}$. Hypothetically putting this value in Equation (2) gives the ratio 4.5×10^3 , which is not far off Q as calculated previously, and in the right direction for the loaded cavity. Though this phenomenon suggests a technique developed by Von Hippel, et al ⁽¹²⁾, which determines microwave dielectric characteristics of a material in waveguide from a probe in a slotted line cavity, the probe was used to determine standing waves in the cavity and the relationship may be superficial. Any interpretation of the data so far accumulated must be very tentative, however. As this is written, we do not know what theoretical connections exist, if any, between frequency-dependent absorption differences and differences due to sample location.

It is clear that when the sample tube is located at an absorption maximum for one solution, corresponding to the X-axis of Fig. 22, ordinates of the reflected microwave signal have different amplitudes for other solutions at the same temperature. Acetone and glycerol have given consistently larger and smaller respective amplitudes. There are small but consistent differences between the ordinates of water-based solutions and those for water alone. In Fig. 16, a solution of 1 g/60 ml, bovine serum albumin in 0.1N Na Cl, is compared with water alone at 21° and 25° C. At both temperatures and a summation of 150 analyzer sweeps (10 msec/cm) per/ordinate, the amplitude of the b. s. a. (bovine serum albumin) solution is greater. Since for equal

volumes the liquid which exhibits maximum absorption will produce a minimum reflected signal, the data is taken to mean that the absorption of water is greater in this case.

Greater absorption of water in an equal sample volume is believed to be linked directly to the greater number of free dipoles in the water solution ⁽¹¹⁾. The loss of the dielectric goes through its maximum at microwave frequencies, hence as the molecule exhibits relaxation, induced molecular polarizations result in a maximum absorption of the incident energy, and the microwave power dissipated must be subtracted from the reflected cavity signal. In a solution, the reduced number of free dipoles is due both to displacement of water by the solute molecule and to irrotational binding.

Data and time to make the labored mathematical approximations for displacement of water by the solute molecule were obviously lacking at this preliminary stage of the investigation. We therefore, added to the solution a molecule which might be expected to replace water at binding sites, thus liberate the attached dipoles and result in an absorption increase out of proportion to the space taken up by the new molecule. If it is assumed that no frequency shifts associate with the respective absorption maxima, data from Figs. 17 and 18 may be taken to indicate that the addition of a barbiturate to fairly concentrated solutions of b. s. a. (2g/17ml, 0.1N Na Cl) not only released enough bound water to compensate for its additional solution space but also may have itself exhibited relaxation, since the ordinate amplitudes decreased in the direction of water alone. Though this might be substantiated by the relatively small size of the barbiturate molecule and the displacement

of irrotationally-bound groups by rotationally-bound ones, we have no information at this time about the microwave spectrum of the barbiturate and we lack corroborative data for specific binding mechanisms between the solute molecules. So far only amplitude differences have been studied, and these at the absorption maximum for water. Thus this data must be considered as indicative rather than qualitative, although the area of investigation suggested is a very pertinent one.

It is well-known that the critical wavelengths associated with the microwave relaxation of water are a function of temperature. For water circulated into a fixed cavity location at increasing temperatures, a consistent amplitude difference in the display ordinates would link^k these ordinates directly with its known spectral characteristics and serve as an additional cross-check against the possibility of the response being spurious. In Fig. 19 the ordinates from center to right decrease in amplitude for each increase in the temperature between 9° and 75° C, with good agreement for a second 9° ordinate following the series. The direction of the amplitude change is linked to frequency shifts and undoubtedly associated with heightened thermal energy in the molecule. Thus the interpretation must again be restricted to mean only that consistent change does exist. The ordinates to the left of center increase as the temperature in the sample tube falls and the solution loses heat.

In Figs. 20 and 21, the output of the oscillator was modulated with its internal 1 KHz source to investigate possible differences in the side-band products between the various solutions. In Fig. 20, single sweeps were triggered for the empty tube and for glycerol at dispersion and resolution

settings of 20KHz/cm. There is a clear difference in absorption amplitudes, but the modulation products in the trace are similar. In Fig. 21, taken at dispersion/resolution settings of 10KHz/5KHz/cm, single sweeps again show amplitude differences between b. s. a. and water but similar modulation products. In both cases, the data was taken at reflection maxima for the empty tube and water. These maxima occur immediately adjacent to the absorption maximum position, as in Fig. 22, and the choice of a reflection maximum generally inverts the results taken at the absorption maximum (i. e. , water appears at a higher amplitude than solutions). We are trying to apply the narrowest analyzer bandwidths to the sideband products to give maximum resolution to the trace. This has been difficult so far because of oscillator and sample temperature variability, but the installation of the feed-back temperature control system outlined in the previous report and external levelling at the source should permit this analysis.

Future Work:

Preliminary findings have suggested a variety of further experiments and have pointed up deficiencies in the existing instrumentation. Future experiments will be designed to clarify the amplitude changes we have seen so far and to associate the relevant frequency data by determining the oscillator frequency at which each absorption maximum occurs. From among the number of protein molecules which have been studied at microwave frequencies, we will examine horse hemoglobin and gelatin in the near future. We propose to extract red cell ghosts from whole blood, then to determine the effects of specific molecules on the microwave absorption of water-based solutions of these ghosts. Given sufficient instrument sensitivity, such a study could differentiate between those pharmacological agents which affect the water-binding properties of the solute molecules and those which merely displace water in the solution, thus clarifying the mechanisms underlying membrane association for these agents. We will then consider nerve cell preparations in similar studies, attempting to answer how such agents as anaesthetics relate to bound-water measurements of solutions of cellular and subcellular structures. This program will reveal both the capability and the limitations of the instrumentation, as it becomes refined to meet new experimental demands.

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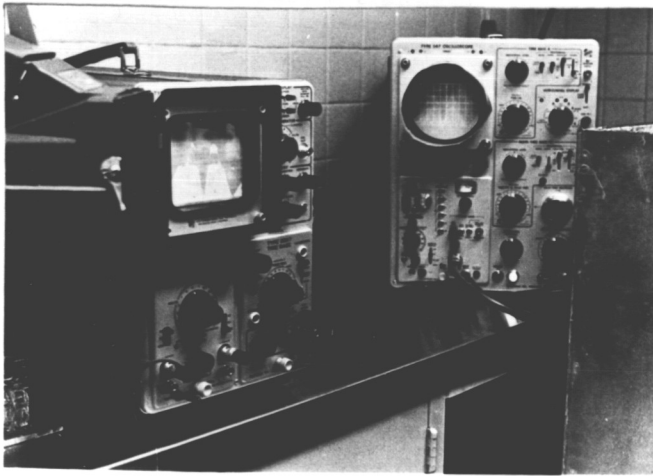


Fig. 1 Spectrum analyser and recording oscilloscopes.

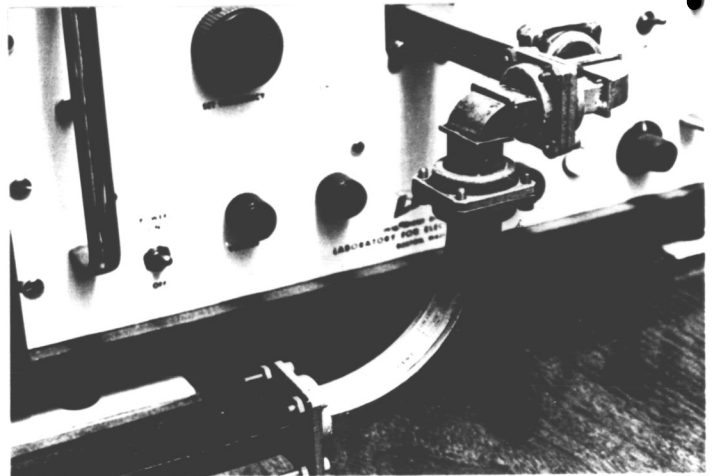


Fig. 4 10 db attenuator and waveguide transmission line at source.

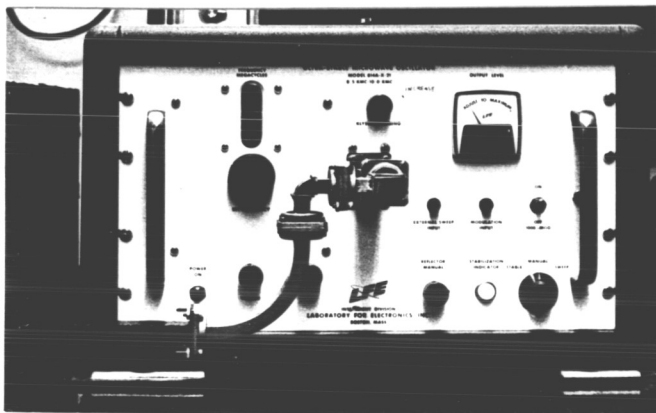


Fig. 2 Microwave Sweep Oscillator.



Fig. 5 Two-directional coupler, 6 db attenuator, and waveguide to coax adapter inside environmental chamber.

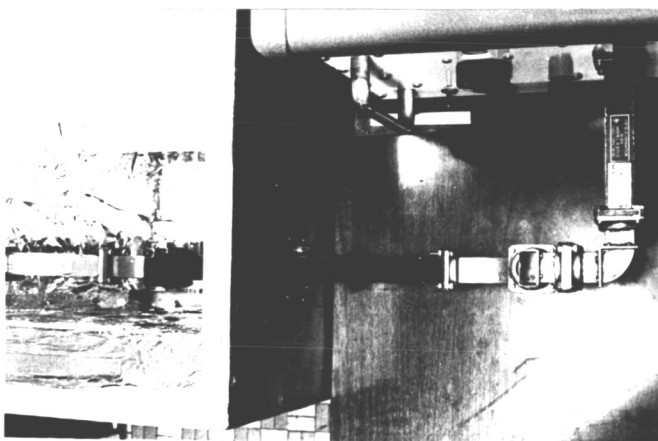


Fig. 3 Top view of transmission line from source to cavity through environmental chamber.

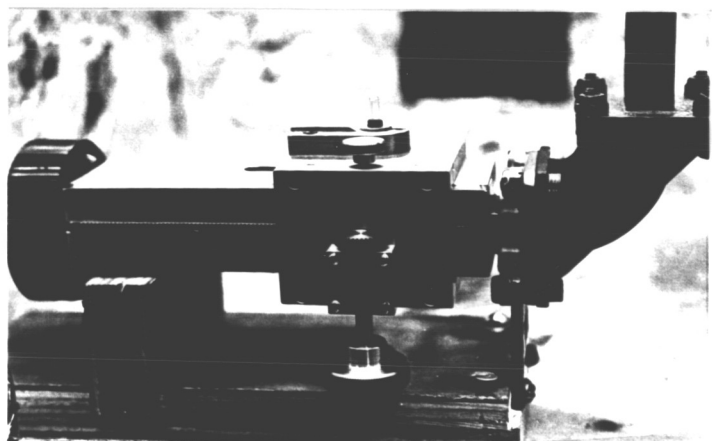


Fig. 6 Cavity mounted on chamber floor with sample capillary tube in place.

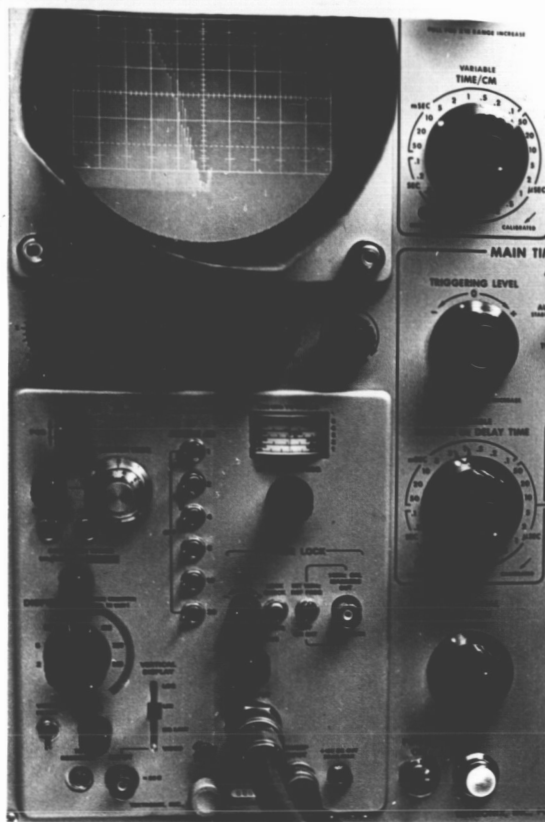


Fig. 7 Tektronix # 1L30 Spectrum Analyser plug-in installed in # 547 Oscilloscope.



Fig. 8 Sharp absorption cusp of tuned empty cavity, as photographed on Tektronix # 564 Storage Oscilloscope. Trace shows volts vs. frequency.

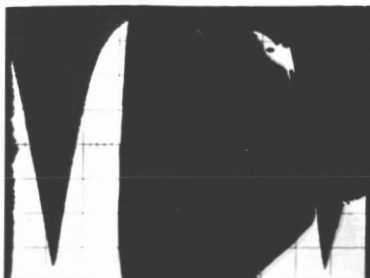


Fig. 9 Frequency displacement from the dielectric loss of glass capillary introduced into the cavity at a maximum absorption node. Sweep of 0.5 MHz/cm shows $f_o - f_g = 3.75$ MHz.

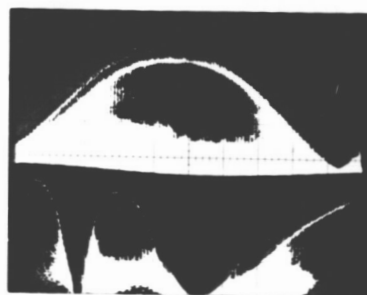


Fig. 10 Lower trace shows sharp absorption dip of empty glass tube. Upper trace shows reflection broadening when the capillary is filled with water. Sweep of 1 MHz/cm shows absorption peak shift of 2.75 MHz.

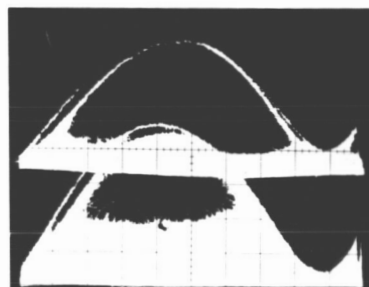


Fig. 11 Upper trace shows broadened reflection of water. Lower trace shows the sharper reflection of a concentrated solution of egg albumin and glycerol in water. Absorption peak shift: approximately 1 MHz.

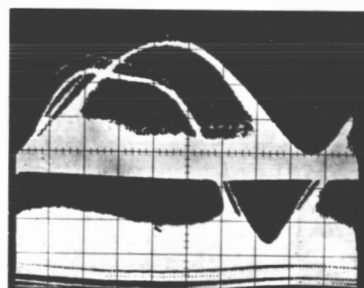


Fig. 12 Upper trace shows water. Lower trace shows intermediate solution of egg albumin and glycerol in water. Absorption peak shift: < 1 MHz.

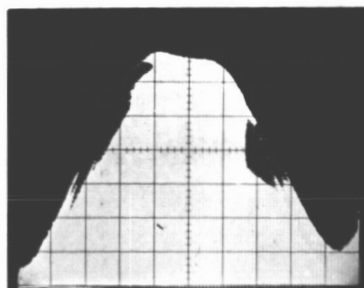


Fig. 13 Solid trace shows absorption of water in a carefully tuned cavity. Broken trace of spiked shows absorption due to a dilute egg albumin-glycerol solution. Difference is hardly discernable at this magnification.

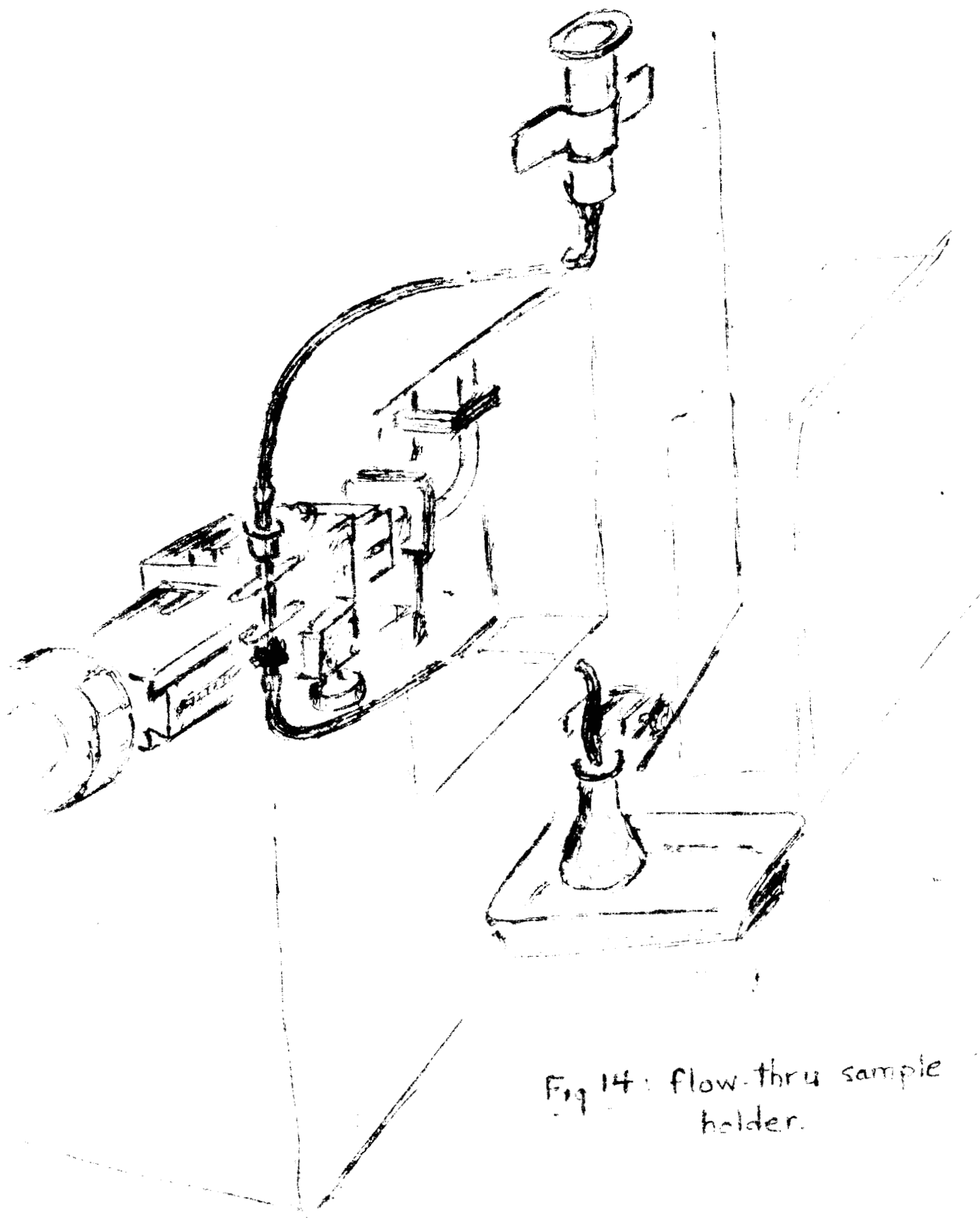


Fig 14: flow-thru sample holder.

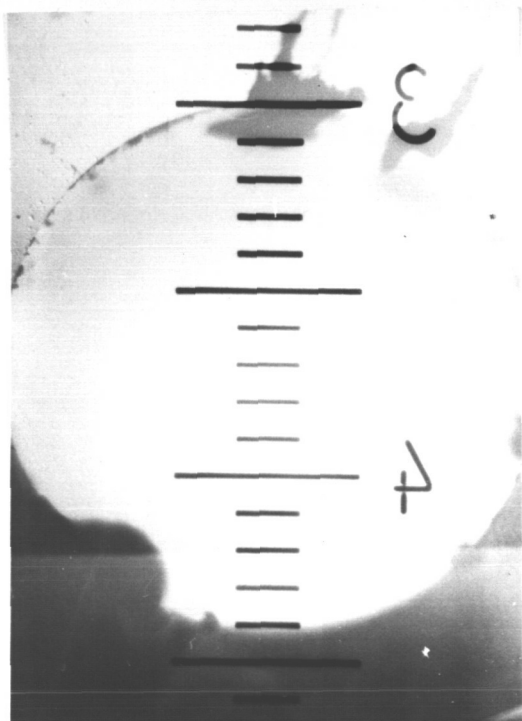


Fig. 15. Microphotograph of sample tube inner cross-section with 0.1 mm/div. graticule.

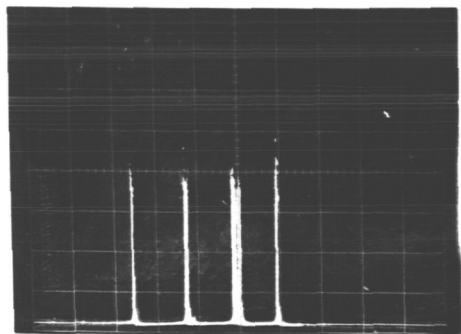


Fig. 16. Left to right: b.s.a., water, water, b.s.a. (b.s.a.=bovine serum albumin). In first two ordinates, solutions 5° warmer.

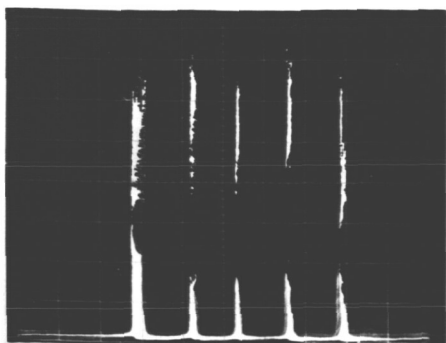


Fig. 17. Left to right: b.s.a. with barbiturate, b.s.a., b.s.a. with barbiturate, b.s.a., water.

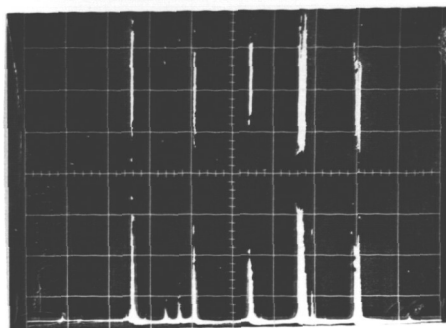


Fig. 18. Left to right: b.s.a., water, b.s.a. with barbiturate, b.s.a., water.

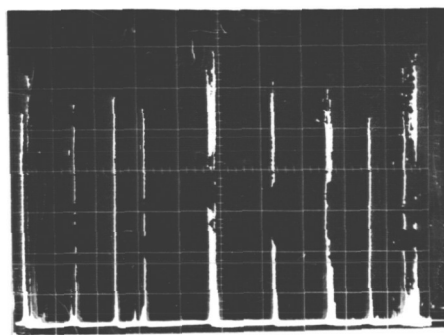


Fig. 19. Water vs temperature. Center to right: 9°, 27°, 42°, 75°, 9°. At left ordinates change as warm solution loses heat in sample tube.

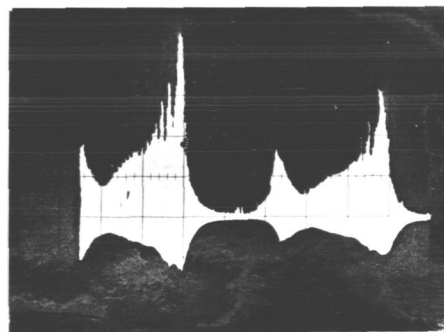


Fig. 20. Absorption of empty tube, left, and egg albumin in glycerol, right, for modulated microwaves at dispersion/resolution=20 KHz/cm.

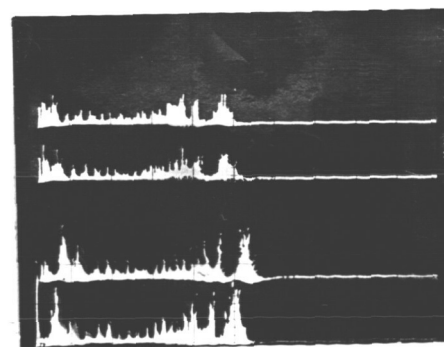


Fig. 21. Single traces of b.s.a. (upper traces) and water (lower traces) for modulated microwaves at dispersion/resolution = 10 KHz/5KHz/cm.

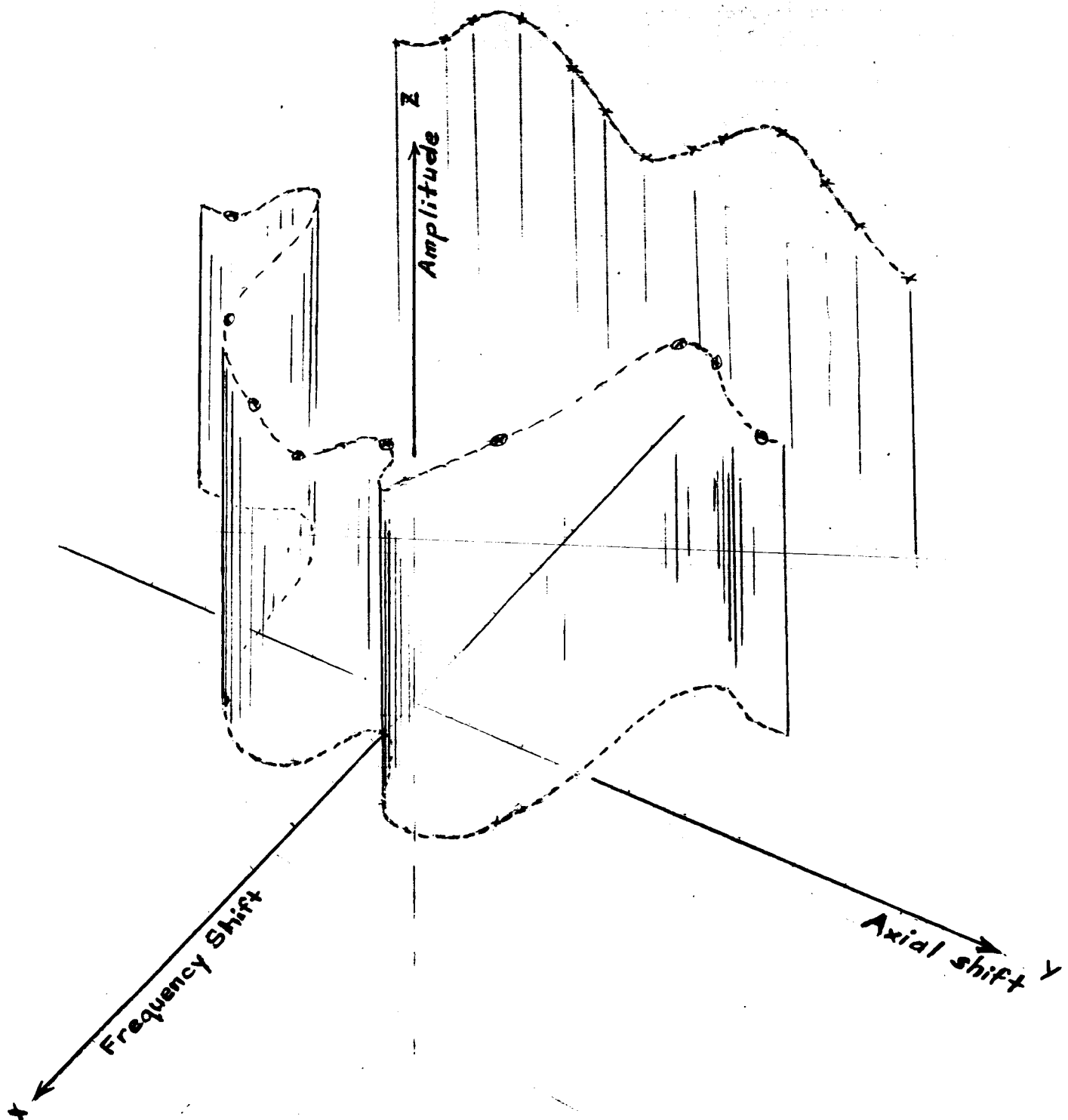


Fig. 22 . Three-dimensional graph of Δf and ΔP with axial shift of sample tube. Projections of Δf on X-Y plane and ΔP parallel to Y-Z plane.